

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	261
MMRRC Strain Name	STOCK Tg(Igf1-EGFP)CO18Gsat/Mmnc
Gene Name(s)	Insulin-like growth factor 1 (Igf1), EGFP
Breeding Protocol(s)	Backcross to Swiss Webster (Taconic)
Protocol Date	1/28/13

MMRRC# 261 PCR Reaction

	<u>1X</u>
ddH ₂ O	11
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM WT Primers F/R	1/1
10uM Mut Primers F/R	1/1
Taq	0.5
DNA	2

Note: This is a 1, 4 Primer Reaction

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 60°C for 30 sec
 Step 4: 72°C for 30 sec
 Step 2 to 4 Cycles: 35
 Step 5: 72°C for 7 min

Taq: **Apex Taq and Chromataq 5X Buffer**

Bands:

MUTANT: 332bp

WT: 232bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer

WT-F (261): TGATGAGAAGGAGTACCAAGTGCGAGT

WT-R (261): CGAACTGAAGAGCATCCACCAGCT

Mut-F (261): TGATGAGAAGGAGTACCAAGTGCGAGTC

Mut-R(261): AAGCACTGCACGCCGTAGGTCA

Run on 2.0% agarose gel in TAE.